REGULATION OF MATING AND MEIOSIS IN YEAST BY THE MATING-TYPE REGION

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Manuscript received July 7, 1975
Revised copy received October 3, 1975

ABSTRACT

A supposed sporulation-deficient mutation of *Saccharomyces cerevisiae* is found to affect mating in haploids and in diploids, and to be inseparable from the mating-type locus by recombination. The mutation is regarded as a defective **a** allele and is designated **a***. This is confirmed by its dominance relations in diploids, triploids, and tetraploids. Tetrad analysis of tetraploids and of their sporulating diploid progeny suggests the existence of an additional locus, *RME*, which regulates sporulation in yeast strains that can mate. Thus the recessive homozygous constitution *rme/rme* enables the diploids **a***/α, **a**/**a***, and α/α to go through meiosis. Haploids carrying *rme* show apparent premeiotic DNA replication in sporulation conditions. This new regulatory locus is linked to the centromere of the mating-type chromosome, and its two alleles, *rme* and *RME*, are found among standard laboratory strains.

THE mating-type locus in yeast regulates the alternation between the haplophase and the diplophase. Mating between two cells is accomplished only if they are of different mating types (**a** $\times$ α, **aa** $\times$ $\alpha\alpha$, **aa** $\times$ α, etc). The ploidy of the cells does not play a role in mating (ROMAN and SANDS 1953). The meiotic process can start when the cell is heterozygous for the mating- type locus, i.e. **a**/α. Normal **a**/α diploids will accomplish meiosis and spore formation, while **a**/α disomic haploids will enter meiosis but will not form viable ascospores (ROTH and FOGEL 1971).

One of the main approaches to the study of the function and structure of the mating-type locus is to obtain mutations which affect mating and/or meiosis. HAWTHORNE (1963) selected for a haploid that was able to mate with other haploids from the same mating type. He obtained a mutation from the α allele to the **a** allele. This mutation was associated with a recessive lethal deletion in the same region. MACKAY and MANNEY (1974a, b) selected for non-mating haploids. They characterized at least five loci. The loci were defined through complementation and genetic mapping, and the mutants differed in their ability to produce sex hormone, to respond to the hormone of the opposite mating type, to mate at low frequency and to sporulate. GERLACH (1974) obtained a recessive mutation that was unlinked to the mating-type locus and enabled **aa** or $\alpha\alpha$ diploids to sporulate. HOPPER and HALL (1975) also selected for mutants that enabled **aa** or $\alpha\alpha$ diploids to sporulate. In this way they could identify a mutation

that separated the induction of premeiotic DNA synthesis from regulation by the mating-type locus.

SIMCHEN, PIÑON and SALTS (1972) selected for a dominant mutation that affected sporulation, by inducing it in an **a** haploid strain, and mating it to a normal α. The diploid, designated 132, did not sporulate but started certain meiotic functions (SIMCHEN, PIÑON and SALTS 1972; PIÑON, SALTS and SIMCHEN 1974). In this work we demonstrate that the sporulation-deficient mutation carried by strain 132 also affects mating, and that it resides at or near the mating-type locus.

MATERIALS AND METHODS

Strains: The heterothallic strains of *Saccharomyces cerevisiae* used in this study, and their genotypes, are listed in Table 1.

TABLE 1

Genotypes of yeast strains

Haploids	Genotype	
320	**a** *ade2 ura3 can1-11 leu1 cyh2-21*	
17-15	same as 320, but carrying an induced sporulation deficiency	
G1466	α *ade2-7 his6*	
G1301	α *ade2 leu1 his7 lys9 cyh2-21*	
G1314	α *ade2 thr3 ura3 leu1 cyh2-21*	
308	α *ade2-R8 met8*	
Tester strains		
310	α *lys1*	
311	**a** *lys1*	
Diploid strains	Origin	Phenotype
131	308 × 320	*ade*$^-$
132	308 × 17-15	*ade*$^-$ *spo*$^-$
Y561	G1466 × 320	*ade*$^-$
Y170	G1466 × 17-15	*ade*$^-$ *spo*$^-$
Y563	G1301 × 320	*ade*$^-$ *leu*$^-$ *cyh*^{r}
Y630	G1301 × 17-15	*ade*$^-$ *leu*$^-$ *cyh*^{r} *spo*$^-$
Y562	G1314 × 320	*ade*$^-$ *ura*$^-$ *leu*$^-$ *cyh*^{r}
Y632	G1314 × 17-15	*ade*$^-$ *ura*$^-$ *leu*$^-$ *cyh*^{r} *spo*$^-$
131-20-2	UV irradiation of a derivative of 131	*ade*$^-$ *ura*$^-$ *can*^{r} **a**-mater
131-20-40	UV irradiation of a derivative of 131	*ade*$^-$ *ura*$^-$ *met*$^-$ *can*^{r} α-mater
Triploid strains		
Y640	17-15 × 131-20-40	*ade*$^-$ *ura*$^-$ *can*^{r}
Tetraploid strains		
Y572	(17-15 × Y170) × 320	*ade*$^-$
Y573	Y170 × 131-20-2	*ade*$^-$

Note: *ade2* that appears in different strains does not necessarily denote the same allele.
Gene symbols indicate mutations and requirements for the following: *ade* (adenine), *ura* (uracil), *leu* (leucine), *his* (histidine), *thr* (threonine), *met* (methionine), *lys* (lysine), *can*^{r} (canavanine resistance), *cyh* (cyclohexamide resistance), **a** or α mating type, *spo*$^-$ (sporulation deficiency).

Diploids that were homozygous for the mating-type locus were obtained as mating colonies following UV irradiation and mitotic recombination. Strain 131 was irradiated and a canavanine-resistant colony was selected, that also required uracil. This *ura⁻can*^{r} derivative was irradiated again to give the **aa** diploid 131–20–2 (PIÑON, SALTS and SIMCHEN 1974). Similarly, this treatment gave a *ura⁻met⁻can*^{r} derivative, which was again irradiated to give the $\alpha\alpha$ diploid 131–20–40.

A triploid strain designated Y640 was obtained from a cross between 17–15 and 131–20–40 (see Table 1) and was selected as a colony growing on medium lacking methionine and leucine.

The tetraploid Y572 was obtained in two steps of zygote isolation. First, we isolated a triploid zygote from the mating of 17–15 to strain Y170; this triploid could mate with **a** strains, so we mated it with 320 and isolated a zygote to give the tetraploid Y572. The tetrapolid Y573 was constructed by mating strain Y170, which could mate with **a** strains, to strain 131–20–2, and isolating a zygote with a micromanipulator.

Media: PSP2—acetate growth medium: yeast nitrogen base (without amino acids) 6.7 g, yeast extract 1.0 g, potassium acetate 10.0 g in 1 liter 0.05 M potassium phthalate buffer (pH 5.0). This medium was supplemented with adenine, uracil (40 μg/ml), leucine and histidine (20 μg/ml), as required. SPM—sporulation medium. potassium acetate 3.0 g, raffinose 0.2 g in 1 liter distilled water. YEP: 20 g glucose, 20 g bacto peptone, 10 g yeast extract in 1 liter distilled water. MIN—minimal medium: yeast nitrogen base (without amino acids) 6.7 g, glucose 20 g in 1 liter distilled water. Media were solidified with 1.5%agar.

Vegetative growth and sporulation conditions: Details are given by SIMCHEN, PIÑON and SALTS (1972).

Mating-type analysis: Auxotrophic colonies to be examined were replicated together with a lawn of one of the tester strains, 310 or 311, on YEP plates. After one day of mating, the plates were replicated onto MIN plates. The ability of a colony to grow on MIN medium indicated that mating occurred with the tester strain. Another method was by recognizing zygote-shaped cells in the phase microscope. The examination was done after 2 hours of incubation (shaking, 30°, YEP media) of a mixture of a tester strain with inoculum from the tested colony.

Kinetics of mating: Strains were grown in liquid YEP medium at 30° with shaking to about 5×10^6 cells/ml. The cells were washed twice in water and resuspended in YEP at a concentration of 5×10^6 cells/ml. The two strains to be mated, one of which was usually a tester strain, were mixed in approximately equal proportions and reincubated at 30° with gentle shaking. At hourly intervals, samples were plated on YEP and on MIN plates. Percent mating was calculated by comparing the number of colonies grown on minimal media to that on YEP medium.

Premeiotic DNA synthesis: DNA synthesis in SPM medium was measured by prelabeling (in PSP2) the culture with uracil-2-C^{14}, and examining the amount of label that was incorporated into DNA at times after transfer to "cold" SPM. Details have been described previously (SIMCHEN, PIÑON and SALTS 1972).

RESULTS

The ability of diploid strains to mate and to sporulate

The haploid strain 17–15, which contains the sporulation-deficient mutation, and its normal progenitor, 320, were mated to a large number of α haploids. The resulting diploids were examined for their ability to mate and to sporulate. The results obtained for some of these diploids are summarized in Table 2.

Diploids that were produced by 320 were normal, i.e. they were non-maters and sporulated with high frequency. Diploids produced by 17–15 behaved differently: they mated as if they were α/α diploids. Some of the mater diploids were sporulation-negative, and some had a low frequency of sporulation, depending

TABLE 2

The ability of the different diploid strains to mate and to sporulate

Strain	Mating with a	Mating with α	Percent asci at 48 hours
308 × 320	—	—	80%
308 × 17–15	+	—	0%
G1466 × 320	—	—	70%
G1466 × 17–15	+	—	0%
G1301 × 320	—	—	85%
G1301 × 17–15	+	—	26%
G1314 × 320	—	—	75%
G1314 × 17–15	+	—	19%

upon the α strain used. Thus, G1301 and G1314 probably contain a genetic factor, or modifier, that permits the sporulation of the diploids Y630 (G1301 × 17–15) and Y632 (G1314 × 17–15) in spite of the fact that these diploids can mate with **a**. This factor might be dominant, i.e., might permit sporulation in a heterozygous diploid, or it might be recessive. If the factor in G1301 and G1314 is recessive, then strain 17–15 must also contain it. It would then follow that diploids Y630 and Y632 are homozygous for this factor and can sporulate, while diploid Y170 is heterozygous and therefore cannot sporulate.

Mating behavior of haploid strains

The results described above indicate that the sporulation-deficient mutation in 17–15 also affects mating behavior of the diploid strains derived from it. Sporulation ability of a strain can only be checked in diploids, while mating ability can be examined in haploids, where it is not affected by the other haploid genome.

Figure 1 gives a respresentative experiment on the kinetics of mating in the haploid **a** strain. (For details of procedure, see MATERIALS AND METHODS). We can see that both strains, 320 and 17–15, do not mate at all with an **a** tester strain 311. Both strains do mate with the tester strain 310, but 17–15 is less efficient in mating than strain 320.

Mapping of the mutation in strain 17–15

The existence of diploids that carry the mutation affecting sporulation and that still sporulate enabled us to determine the linkage relationship between this mutation, its putative modifier (from strain G1301), and the mating-type locus.

Strain Y630 (G1301 × 17–15) showed 30% asci at 72 hrs in sporulation conditions. Samples were dissected and the spores from each tetrad were grown into colonies and analyzed. Segregation at the mating-type locus could be directly analyzed in the haploid colonies. The mutations in strains 17–15 and G1301 could be recognized only in diploids; therefore, we mated the segregants to the following strains: G1466, 320 and 17–15.

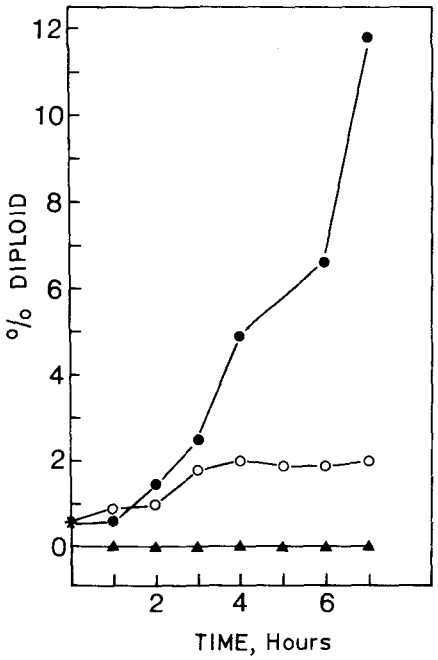

FIGURE 1.—Mating kinetics of strain 17–15 and its normal parent strain 320. Strains 320 and 17–15 were each mixed with strain 310 or 311 at a concentration of 5×10^6 cells/ml and incubated in a shaking bath at 30°. At hour intervals samples plated on MIN and YEP media. Percent mating was calculated from the number of colonies grown on MIN compared with the number of colonies grown on YEP.

● 320 × 310, ○ 17–15 × 310, ▲ 320 × 311 and 17–15 × 311

We isolated 10 tetrads with 4 viable spores each that gave a segregation pattern of 2**a**:2α, and 8 tetrads with only 3 spores each that gave either 2**a**:1α or 1**a**:2α segregation. From these tetrads plus an additional 32 tetrads with one and two viable spores, 63 colonies were classified as **a** mating-type (designated **a**-maters) and 48 were classified as α mating-type (designated α-maters). The 63 **a**-mater colonies were mated to the α strain G1466. Diploids were isolated either by prototroph selection or by micromanipulation. All diploids were α-maters and gave no sporulation, implying that all the **a**-mater haploid segregants carried the mutation from 17–15. The 48 α-mater colonies obtained from the dissection were mated to strain 320, and gave diploids that were nonmaters and sporulated normally. This implied that the α-mater segregants did not carry the mutation from 17–15. The same α segregants were mated also to strain 17–15 and gave 48 α-mater diploids that could sporulate; thus they contained the modifier from G1301.

The segregants were of two types only, like their parents. This indicates that the mutation in strain 17–15 is tightly linked to the **a** allele, and the modifier in strain G1301 is either dominant and closely linked to the α allele, or recessive in a homozygous condition.

Dominance relationship

Originally, the mutation in strain 132 was reported as a dominant mutation (SIMCHEN, PIÑON and SALTS 1972). In view of the results reported here, we postulate that the dominance is due to an interaction between a defective **a** allele and a normal α. Thus, the diploid strains Y170 (17–15 × G1466) and 132 (17–15 × 308) are α-maters and sporulation-deficient due to a defective **a** allele designated **a***, that cannot "inactivate" the normal α allele. (This is based on the notion that in a normal **a**/α diploid the two alleles of the mating-type locus inactivate each other and therefore the diploid cannot mate. However, this is not the only interpretation of the mutual relationship between **a** and α in diploids.) The presence of an active α allele in the cell does not allow meiosis to take place. We expect the **a*** mating-type allele in 17–15 to be "dominant" with regard to any number of α alleles in the genome, but "recessive" in the presence of normal **a**. Thus, we constructed a triploid strain that contained **a*** from strain 17–15, and two normal α alleles that originated from strain 308. This **a***$\alpha\alpha$ triploid, designated Y640 (see MATERIALS AND METHODS), mated as an α and gave no spores at 72 hrs. Furthermore, we constructed two tetraploid strains. The first was of the **a***$\mathbf{aa}\alpha$ genotype (Y573) and the second was **a***$\mathbf{a}^*\mathbf{a}\alpha$ (Y572). Both tetraploid strains were nonmaters and gave about 30% asci at 48 hrs.

The results obtained agree with our working hypothesis that the lack of sporulation in **a***α diploids is due to the presence of an active α allele.

Tetrad analysis of the tetraploids

Our model assumes that **aa*** and **a***$\mathbf{a}^*$ genotypes will be **a**-maters and will not sporulate. In order to verify this assumption, we sporulated both tetraploid strains mentioned above, dissected tetrads and analyzed them for mating type and for their ability to sporulate. The analysis could also verify the postulated genotypes of the tetraploid strains.

We expected the tetraploid **a***$\mathbf{aa}\alpha$ to give rise to the following type of tetrads (*nm* stands for nonmater, *spo*$^-$ and *spo*$^+$ stand for sporulation deficiency and proficiency, respectively):

Type I		Type II		Type III	
Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype
$\mathbf{a}\alpha$	*nm spo*$^+$	**aa**	**a** *spo*$^-$	$\mathbf{a}\alpha$	*nm spo*$^+$
$\mathbf{a}\alpha$	*nm spo*$^+$	**aa**	**a** *spo*$^-$	$\mathbf{a}^*\alpha$	α *spo*$^-$
aa*	**a** *spo*$^-$	$\mathbf{a}^*\alpha$	α *spo*$^-$	**aa***	**a** *spo*$^-$
aa*	**a** *spo*$^-$	$\mathbf{a}^*\alpha$	α *spo*$^-$	**aa**	**a** *spo*$^-$

The tetraploid **a***$\mathbf{a}^*\mathbf{a}\alpha$ is expected to give the following tetrads:

Type I		Type II		Type III	
Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype
aa*	**a** *spo*$^-$	$\mathbf{a}^*\mathbf{a}^*$	**a** *spo*$^-$	$\mathbf{a}^*\mathbf{a}^*$	**a** *spo*$^-$
aa*	**a** *spo*$^-$	$\mathbf{a}^*\mathbf{a}^*$	**a** *spo*$^-$	**aa***	**a** *spo*$^-$
$\mathbf{a}^*\alpha$	α *spo*$^-$	$\mathbf{a}\alpha$	*nm spo*$^+$	$\mathbf{a}^*\alpha$	α *spo*$^-$
$\mathbf{a}^*\alpha$	α *spo*$^-$	$\mathbf{a}\alpha$	*nm spo*$^+$	$\mathbf{a}\alpha$	*nm spo*$^+$

The results of the actual analysis are given in Tables 3 and 4.

Judged from mating ability, the alleles of the mating-type locus segregated exactly as expected, giving the phenotypes: 2*nm*:2**a**, 1*nm*:1α:2**a**, 2**a**:2α. On the other hand, among the diploid segregants, the ability to sporulate segregated almost independently from the mating-type locus. Some of the postulated **a***α, **aa***, **a*****a*** genotypes were actually sporulating.

The discrepancy between our previous assumption concerning **a*** and the sporulation behavior of the segregants can be resolved by assuming the segregation of another gene, *RME* (*regulator of meiosis*). Strain 320 is assumed to contain a recessive mutation at the *RME* locus, designated *rme*. A homozygous condition of *rme* is assumed to enable the diploid cell to sporulate regardless of the situation at the mating-type locus. The dominant allele at the *regulator of meiosis* locus is designated *RME*. We think that *RME* is located on the same chromosome as the mating-type locus, either very close to the centromere or on the other arm of the same chromosome. The location of *RME* is based on the fact that it was able to segregate, but not independently from the mating-type locus, as can be noticed from our inability to get tetrads that segregate 2**a** *spo*$^-$:2α *spo*$^+$ (Tables 3 and 4). We assume the following genotypes for the different strains. Strain 320: *rme* **a**, strain 17–15: *rme* **a***, strain 308: *RME* α, and strain G1466: *RME* α.

TABLE 3

Tetrad analysis of strain **a*****aa**α

Type of tetrad	Mating type	Sporulation at 72 hrs	No. of tetrads isolated	Postulated genotype †	Postulated genotype §
I	**a**	—		**aa***	*RME* **a**/*rme* **a***
	a	—	2	**aa***	*RME* **a**/*rme* **a***
	nm	+		**a**α	*RME* α/*rme* **a**
	nm	+		**a**α	*RME* α/*rme* **a**
II	**a**	—		**aa** or *vice versa*	*RME* **a**/*rme* **a**
	a	—	2	**aa*** or *vice versa*	*RME* **a**/*rme* **a***
	nm	+		**a**α	*RME* α/*rme* **a**
	α	—		**a***α	*RME* α/*rme* **a***
III	**a**	—		**aa** or *vice versa*	*RME* **a**/*rme* **a**
	a	—	1	**aa*** or *vice versa*	*rme* **a**/*RME* **a***
	nm	+		**a**α	*RME* α/*RME* **a**
	α	+		**a***α	*rme* α/*rme* **a***
IV	**a**	—		**aa** or *vice versa*	*RME* **a**/*rme* **a**
	a	+	1	**aa*** or *vice versa*	*rme* **a**/*rme* **a***
	nm	+		**a**α	*RME* α/*RME* **a**
	α	—		**a***α	*rme* α/*RME* **a***

† Based only on mating ability.
§ New model, based on sporulation as well as mating.
Note: The postulated genotype of the tetraploid is *rme* **a**/*RME* **a**/*rme* **a***/*RME* α.

TABLE 4

Tetrad analysis of strain **a*a*a**α

Type of tetrad	Mating type	Sporulation at 72 hrs	No. of tetrads isolated	Postulated genotype †	Postulated genotype §
I	**a**	—		**aa***	*RME* **a**/*rme* **a***
	a	+	6	**aa***	*rme* **a**/*rme* **a***
	α	+		**a***α	*rme* α/*rme* **a***
	α	—		**a***α	*RME* α/*rme* **a***
II	**a**	—		**aa*** } or *vice*	
	a	—	1	**a*a*** } *versa*	
	α	—		**a***α	
	nm	+		**a**α	
III	**a**	—		**aa*** } or *vice*	*RME* **a***/*rme* **a***
	a	+	1	**a*a*** } *versa*	*rme* **a***/*rme* **a**
	α	+		**a***α	*rme* **a***/*rme* α
	nm	+		**a**α	*RME* α/*rme* **a**
IV	**a**	+		**aa***	*rme* **a**/*rme* **a***
	a	+	4	**aa***	*rme* **a**/*rme* **a***
	α	—		**a***α	*RME* α/*rme* **a***
	α	—		**a***α	*RME* α/*rme* **a***
V	*nm*	—		**a**α	*rme* **a***/*rme* **a***
	α	+	1	**a***α	*rme* α/*rme* α
	a	+		**aa*** } or *vice*	*rme* **a**/*rme* **a***
	a	—		**aa*** } *versa*	*RME* **a**/*RME* **a***

†, § See footnotes to Table 3.
The postulated genotype of the tetraploid is *rme* **a***/*rme* **a***/*rme* **a**/*RME* α.

This model explains the phenotypes of diploid segregants obtained in the tetrad analysis of the tetraploid strains.

Let us now trace back the origin of the tetraploids. Strain Y573-**a*aa**α was constructed in the following manner:

crossing: 320(*rme* **a**) × 308(*RME* α)

↓

diploid 131(*rme* **a**/*RME* α)

↓ UV treatment to induce mitotic recombination between **a** and the centromere

131–20–2(*rme* **a**/*RME***a**) × Y170(*rme* **a***/*RME* α)

↓

Y573-tetraploid: *rme* **a**/*RME* **a**/*rme* **a***/*RME* α

Strain Y572-**a*a*a**α is *rme* **a***/*rme* **a***/*rme* **a**/*RME* α, and was constructed, as mentioned in MATERIALS AND METHODS, by mating progressively one strain to the other.

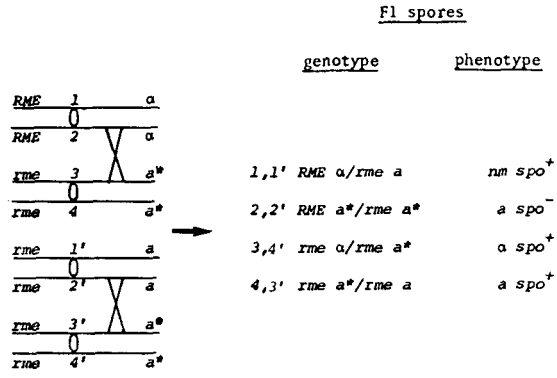

FIGURE 2.—Illustration of pairing and crossing over resulting in segregation of type III tetrad (Table 4) of strain **a*a*a**α. The numbers indicate centromeres. 1–4: first bivalent, 1′–4′: second bivalent.

In order to test our model we had to analyze two rounds of sporulation. First, we sporulated the tetraploid strain, dissected asci and isolated the F_1 diploid segregants, and then examined the latter for mating and sporulation ability. The second step was to induce sporulation of the diploid segregants that were able to do so, to dissect their asci and isolate the F_2 haploid spores. The F_2 haploid progeny were analyzed by mating them to the three original strains: G1466 (*RME* α), 320 (*rme* **a**), 17–15 (*rme* **a***). We present here the detailed analysis of one type of tetrad from Table 4. Similar data for the other types of tetrads from Tables 3 and 4 are given in the APPENDIX.

A type III tetrad of strain **a*a*a**α (Table 4) gave the segregation 1**a** *spo*$^-$: 1**a** *spo*$^+$: 1α *spo*$^+$: *nm spo*$^+$. Such a segregation is expected from bivalent pairing and a crossover in each bivalent, as demonstrated in Figure 2. (The results of ROMAN, PHILLIPS and SANDS (1955) indicate that for chromosome *III* of tetraploid *S. cerevisiae*, pairing in bivalents occurs most frequently).

The three F_1 diploid sporulating segregants were further examined. Results of tetrad analysis are given in Table 5. The *nm* progeny segregated 2**a**:2α. The α-mater F_1 progeny segregated also 2**a**:2α, and the **a**-mater F_1 progeny gave only 4**a** spores.

TABLE 5

Tetrad analysis of F_1 segregants from type III tetrad of strain **a*a*a**α

Phenotype of F_1 segregant	No. of 4-spore tetrads examined	Segregation pattern	No. of 3-spore tetrads examined	Segregation pattern
n.m	4	2**a**:2α	3	2**a**:1α
α	2	2**a**:2α	2	1**a**:2α
a	2	4**a**:0α	5	3**a**:0α

Note: Segregation patterns relate to mating phenotypes.

In order to demonstrate the presence of **a** or **a*** in the F_2 generation, the **a**-mater F_1 haploids were mated to strain G1466. As the latter strain is *RME* α, the segregation at the *RME* locus could not be examined in these crosses since *RME* is dominant to *rme*. In any case we expected **a** segregants to give rise to *nm* diploids that could sporulate, and **a*** segregants to give α-mater diploids that could not sporulate. The presence of *rme* could be demonstrated only in α-mater haploids that were mated to strain 17–15 (*rme* **a***). Those diploids were expected to be α-maters but sporulation-proficient. Results of such matings are given in Table 6.

As previously mentioned, the **a**-mater F_1 diploid segregant gave an ascus with four haploid spores that were **a**-maters. Upon mating these F_2 segregants to strain G1466 (*RME* α), the diploids obtained were of two kinds, namely 2 α *spo*$^-$: 2 *nm spo*$^+$. Thus the genotype of the F_1 spore was **aa***.

The α-mater F_1 diploid gave asci that segregated 2**a**:2α. The **a**-mater F_2 segregants gave rise, upon mating with G1466 (*RME* α), to α-mater diploids that could not sporulate. The two α-mater F_2 progeny, when mated to strain 320 (*rme* **a**), gave normal *nm spo*$^+$ diploids, and upon mating to strain 17–15 (*rme* **a***) gave α-mater *spo*$^+$ diploids. These results agree with our presumption that the genotype of the sporulating α-mater F_1 segregant was *rme* **a***/*rme* α.

The *nm* F_1 segregant gave asci that segregated 2**a**:2α. The two **a**-mater colonies from such an ascus gave, upon mating with G1466 (*RME* α), *nm spo*$^+$ diploids

TABLE 6

Mating-type and sporulation ability of different zygotes from F_2 segregants of type III tetrad of strain **a*a*a**α

Phenotype of F_1 segregant	Mating of F_2 spore	Mating type of resulting zygote	Sporulation ability of zygote	Genotype of F_2 segregant	Genotype of F_1 segregant
a	$\mathbf{a}_1\times$G1466	α	—	**a***	
	$\mathbf{a}_2\times$G1466	*nm*	+	**a**	*rme* **a**/*rme* **a***
	$\mathbf{a}_3\times$G1466	α	—	**a***	
	$\mathbf{a}_4\times$G1466	*nm*	+	**a**	
α	$\mathbf{a}_1\times$G1466	α	—	**a***	
	$\mathbf{a}_2\times$G1466	α	—	**a***	*rme* **a***/*rme* α
	$\alpha_3\times$320	*nm*	+	α	
	$\alpha_3\times$17–15	α	+	α	
	$\alpha_4\times$320	*nm*	+	α	
	$\alpha_4\times$17–15	α	+	α	
nm	$\mathbf{a}_1\times$G1466	*nm*	+	**a**	
	$\mathbf{a}_2\times$G1466	*nm*	+	**a**	*rme* **a**/*RME* α
	$\alpha_3\times$320	*nm*	+	α	
	$\alpha_3\times$17–15	α	+	*rme* α	
	$\alpha_4\times$320	*nm*	+	α	
	$\alpha_4\times$17–15	α	—	*rme* α	

The genotypes of the different strains are: G1466 = *RME* α, 320 = *rme* **a**, 17–15 = *rme* **a***.

(Table 6). The two α-maters from the same ascus gave two different diploids when mated to 17–15 (*rme* **a***): one diploid was α-mater *spo*$^+$ and the other was α-mater *spo*$^-$. This means that one spore was *rme* α and the other one was *RME* α. These two haploid segregants, as well as the complementary *RME* **a** and *rme* **a**, are expected to result from a single crossover (at the four-strand stage of meiosis) between the two loci in *RME* α/*rme* **a**.

In other asci obtained from sporulation of the same *nm* F_1 segregant (not given in Table 6), the two α spores were both *RME* α as expected if a crossover did not occur between *RME* and the mating-type locus.

Further results and analysis of F_1 sporulating segregants and their progeny are given in the APPENDIX. These results support the existence of the *RME* locus on the chromosome *III* as postulated above.

The results of the tetraploid segregation show us that recombination can occur between *RME* and the mating-type locus. The following tetrads were obtained (Tables 3 and 4 and Figures 2, A1, A2):

6 tetrads without any crossover;

8 tetrads with one crossover between the centromere and the mating-type locus;

1 tetrad with two crossovers between the centromeres and the mating-type locus—each crossover was in a different bivalent;

2 tetrads with one crossover between *RME* and the centromere and one crossover between the mating type and the centromere, each crossover occurring in a different bivalent.

Premeiotic DNA synthesis

From the foregoing analysis we conclude that diploids that are maters (**aa**, **a***a**, **a***α, αα) and are homozygous for *rme* can sporulate despite their mating-type constitution. We now ask whether meiotic functions can be expressed also in haploid *rme*. The only early biochemical parameter that is expressed in sporulating nonmater diploids and not in nonsporulating mater diploids is premeiotic DNA replication (VEZINHET *et al.* 1974). Premeiotic DNA synthesis can also be easily assessed in haploid cells following the transfer of the cells to sporulation conditions. Results of such assessments for strains G1466 (*RME* α), 320 (*rme* **a**) and 17–15 (*rme* **a***) are given in Figure 3. (For details of label incorporation, see MATERIALS AND METHODS). Strain G1466 (*RME* α) did not show an increase in C^{14} content in DNA, as expected from a "normal" haploid. In contrast, strains 320 (*rme* **a**) and 17–15 (*rme* **a***) showed a marked increase in label content in DNA, in agreement with their postulated *rme* constitution.

In order to verify our model that only *rme* (and not the constitution at the mating-type locus) is responsible for the premeiotic DNA replication, we transferred *rme* to α haploids and examined them for premeiotic DNA synthesis. Type III tetrad of tetraploid **a***a*α gave one F_1 diploid progeny that was *rme* **a**/*RME* α; upon sporulation we obtained (third F_1 segregant in Table 6) four haploid spores with the following postulated genotypes: *rme* **a**, *RME* **a**, *rme* α, *RME* α. The two α haploids were characterized by their ability to sporulate following mating with *rme* **a***. The two **a** spores could not be distinguished

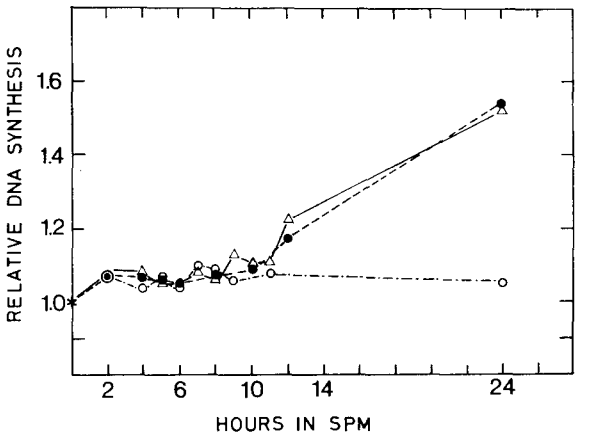

FIGURE 3.—Premeiotic DNA synthesis. Strains G1466, 320 and 17–15 were grown in PSP2 + adenine (40 μg/ml), histidine, leucine (10 μg/ml), uracil (3 μg/ml) + uracil-2-C^{14} (1 μCi/ml, specific activity 60.4 mCi/mM). When cell density reached 1×10^7 cells/ml, the cells were washed twice in distilled water and resuspended in 'cold' SPM. Triplicate samples were removed and C^{14} counts in DNA were determined as described by SIMCHEN, PIÑON and SALTS (1972). The results described here are based on averages of four experiments. At zero time the cell samples had about 3000 cpm in DNA.

○ G1466, △ 320, ● 17–15

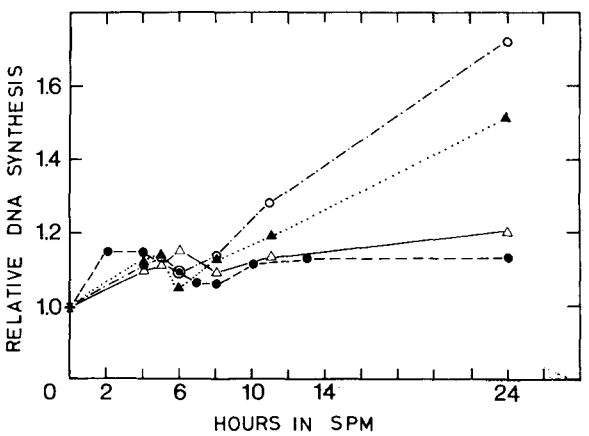

FIGURE 4.—Premeiotic DNA synthesis in four haploid strains segregating as a tetrad from *rme* **a**/*RME* α (third F_1 segregant in Table 6). The strains were grown in PSP2 + adenine (40 μg/ml), histidine, leucine (10 μg/ml), uracil (3 μg/ml) + uracil-2-C^{14} (1 μCi/ml, specific activity 60.4 mCi/mM). When cell density reached 1×10^7 cells/ml, the cells were washed twice in distilled water and resuspended in 'cold' SPM. Triplicate samples were removed and C^{14} counts in DNA were determined as described in SIMCHEN, PIÑON and SALTS (1972). At zero time the cell samples had about 3000 cpm in DNA.

○ $\mathbf{a}_1$, △ $\mathbf{a}_2$, ▲ α_3, ● α_4

from each other. Figure 4 gives the results of DNA synthesis under sporulation conditions for those four haploids. It demonstrates that the *rme* allele operates also in haploids, and that in this particular tetrad, the two segregating loci, **a**/α and *RME/rme*, were presumably separated from each other by a single crossover.

DISCUSSION

In this work we were able to characterize two separate loci for the control of sporulation. The first locus controlling sporulation is the mating-type locus (**a**/α), which also regulates mating. The second locus is the regulator of meiosis (*RME*) which, in homozygous form *rme/rme*, bypasses the control of sporulation by the mating-type locus, probably by enabling cells to start the premeiotic DNA replication.

Strain 17–15 was previously described as containing a dominant mutation for sporulation (SIMCHEN, PIÑON and SALTS 1972). This mutation was revealed by further analysis to affect also mating behavior. The introduction of a presumably single mutation (UV-induced) that simultaneously affected mating and sporulation, lead us to believe that the mating-type locus might have been the target for that specific mutation. And indeed, this mutation was found to be near the mating-type locus, and could not be separated from it. We explain the mutation in strain 17–15 in terms of an abnormal, or mutant, **a*** allele. The **a*** allele in haploids confers low efficiency of mating and in diploids (**a***/α) it cannot "repress" or "inactivate-neutralize" the normal α allele, resulting in α-mater diploids. The existence of an active α product, or the lack of a normal **a**/α complex, inhibits the cell from entering the process of sporulation.

The notion of a complex product of **a** and α is common in the literature (FRIIS and ROMAN 1968; GUTZ 1967). It was previously found that **a***α differs from **aa** and $\alpha\alpha$ in that the former loses viability in sporulation conditions, and in the ability of **a***α to initiate nuclear DNA synthesis (SIMCHEN, SALTS and PIÑON 1973; PIÑON, SALTS and SIMCHEN 1974). These findings also support the formation of an **a**/α complex that "induces" or derepresses" sporulation.

MACKAY and MANNEY (1974a, b) isolated 13 mutants (in both mating-type alleles) that resemble the **a*** allele we described in this paper. They isolated nonmater haploids that upon mating (at low frequency) gave diploids that were maters and were nonsporulating. The diploids mated as though they had the mating type of the parent strain with which the mutant was mated. Indirect evidence from tetraploids that can sporulate (probably of types similar to our **a*****aa**α or **a**α*$\alpha\alpha$) indicated to MACKAY and MANNEY that the mutation was in the mating-type locus itself.

The *RME* locus could be recognized due to the existence of **a***, and through the isolation of *rme/rme* diploids among the segregants of the tetraploids. The *rme* allele enables cells to enter meiosis (or rather the first step, the premeiotic DNA synthesis) regardless of the control of the mating-type locus. We think that *rme* is a regulatory locus and not one of the structural genes for premeiotic DNA replication, because of the recessive nature of the *rme* allele. The exact

mapping of *RME* has not been carried out yet, but our preliminary data (namely from tetraploid segregations) indicated that this locus is located on chromosome *III* (MORTIMER and HAWTHORNE 1973). The position of *RME* on the chromosome is to the left of the mating-type locus, i.e. in the direction of the centromere. *RME* is linked to the centromere, but it is not clear on which chromosomal arm it resides.

Similar mutants that separate the premeiotic DNA replication from the control of the mating-type locus were isolated by HOPPER and HALL (1975) and by HOPPER, KIRSCH and HALL (1975). They characterized in detail one *CSP* mutation (in strain Ap-1-$\alpha\alpha$) and found it to be dominant and unlinked to the mating type. The mutant strain constitutively synthesized DNA under nitrogen starvation. It is, however, different from our *RME* gene in several respects, including its location and dominance. GERLACH's (1974) mutation is, on the other hand, recessive but unlinked to **a**/α.

Our strain G1301 also contained a modifier gene linked to the mating-type locus that enables **a***/α diploids to sporulate. This modification can be easily explained by assuming that G1301 is of *rme* α genotype. Diploid *rme* **a***/*rme* α will sporulate and the ability of sporulation would seem to be linked to the α allele because of the inability to recognize it in the **a*** segregants after mating them to *RME* α. As expected from an *rme* haploid, strain G1301 almost doubled its DNA content in sporulation conditions (unpublished data). Another test to prove the postulated genotype was to isolate **aa** and $\alpha\alpha$ derivates after UV irradiation of a supposed *rme* **a**/*rme* α diploid (that was derived from a cross between 320 and G1301). We expected the homozygotes for the mating-type locus to be able to sporulate because they were also homozygotes for *rme*. Indeed we obtained $\alpha\alpha$ and **aa** derivates that were able to sporulate, and gave 4α or 4**a** segregations in asci, respectively. However, we obtained also other diploids that mated like **aa** or $\alpha\alpha$, sporulated, but showed other segregations. Another group of mater diploids could not sporulate. Thus our allocation of the *rme* α genotype to G1301 is at present only tentative and calls for further study. It should be emphasized, however, that the genetic analysis presented in this report and the postulation of the *RME* locus do not rely in any way on the genetic constitution of G1301.

This work was supported by grant no. 162 from the US-Israel Binational Science Foundation. We thank MRS. JUDITH SHILO for her technical assistance, DR. BEN-ZION DORFMAN for his advice and assistance, and DR. RAPHAEL FALK for his critical reading of the manuscript.

APPENDIX

Tetrad analysis of the tetraploid strains

The results of tetrad analysis of strains **a*****aa**α and **a*****a*****a**α are given in Tables 3 and 4 (see above, RESULTS). Here we present the further analysis of the sporulating diploid segregants in those tables.

Type I tetrad of strains **a*****a*****a**α gave a segregation pattern of 1**a** *spo*$^-$: 1**a** *spo*$^+$: 1α *spo*$^+$: 1α *spo*$^-$. We expected it to arise from bivalent pairing and one crossover, as shown in Figure A1 (i). From the two F_1 segregants that could sporulate, asci were obtained, dissected and analyzed. Results are given in Table A1. The postulated *rme* **a**/*rme* **a*** gave only 4 **a** mater spores in each tetrad. The postulated *rme* α/*rme* **a*** segregated 2**a**:2α. Table A2 contains results of mating

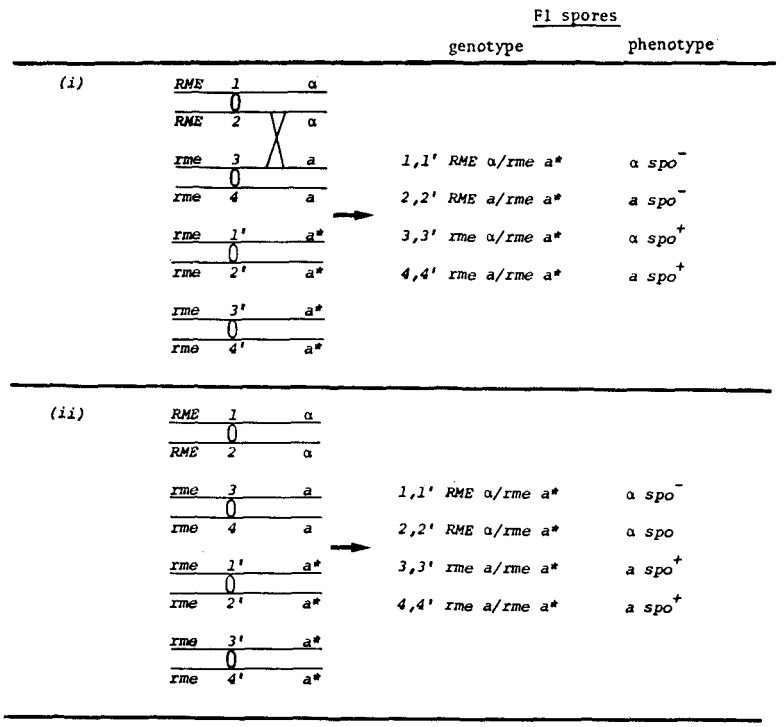

FIGURE A1.—Illustration of pairing and crossing over in the tetraploid strain **a*a*a**α. (i) Type I tetrad. (ii) Type IV tetrad. The numbers indicate centromeres.

tests and sporulation of the diploids produced by the F_2 segregants upon crossing to the three original strains. This analysis shows that the **a**-mater F_1 diploid gave asci of constitution 2**a**:2**a***. The α-mater F_1 diploid progeny gave asci segregating 2**a***:2*rme* α.

Type IV tetrad of strain **a*a*a**α gave a segregation pattern of 2**a** *spo*+ : 2α *spo*−. It resulted from bivalent pairing without exchange as described in Figure A1 (ii). The results of tetrad

TABLE A1

Tetrad analysis of F_1 sporulating segregants from tetrads of strain **a*a*a**α

F_1 tetrad type	Phenotype of F_1 segregants	No. of 4-spore tetrads examined	Segregation pattern	No. of 3-spore tetrads examined	Segregation pattern
I	**a**	2	4**a**:0α	8	3**a**:0α
	α	1	2**a**:2α	1	1**a**:2α
IV	$\mathbf{a}_I$	1	4**a**:0α	4	3**a**:0α
	$\mathbf{a}_{II}$	1	4**a**:0α	4	3**a**:0α
V	**a**	4	4**a**:0α	4	3**a**:0α
	α	9	0**a**:4α	2	0**a**:3α

Note: $\mathbf{a}_I$ and $\mathbf{a}_{II}$ refer to two different segregants of the same ascus. Segregation patterns relate to mating phenotypes.

TABLE A2

Mating-type and sporulation ability of different zygotes from F_2 segregants of tetrads of strain **a*a*a**α

F_1 tetrad type	Phenotype of F_1 segregant	Mating of F_2 spore	Mating type of resulting zygote	Sporulation of zygote	Genotype of F_2 segregant	Genotype of F_1 segregant
I	**a**	$\mathbf{a}_1\times$G1466	α	—	**a***	
		$\mathbf{a}_2\times$G1466	α	—	**a***	*rme* **a**/*rme* **a***
		$\mathbf{a}_3\times$G1466	*nm*	+	**a**	
		$\mathbf{a}_4\times$G1466	*nm*	+	**a**	
	α	$\mathbf{a}_1\times$G1466	α	—	**a***	
		$\mathbf{a}_2\times$G1466	α	—	**a***	*rme* **a***/*rme* α
		$\alpha_3\times$320	*nm*	+	*rme* α	
		$\alpha_3\times$17–15	α	+		
		$\alpha_4\times$320	*nm*	+	*rme* α	
		$\alpha_4\times$17–15	α	+		
IV	$\mathbf{a}_I$	$\mathbf{a}_1\times$G1466	α	—	**a***	
		$\mathbf{a}_2\times$G1466	*nm*	+	**a**	*rme* **a**/*rme* **a***
		$\mathbf{a}_3\times$G1466	α	—	**a***	
		$\mathbf{a}_4\times$G1466	*nm*	+	**a**	
	$\mathbf{a}_{II}$	$\mathbf{a}_1\times$G1466	*nm*	+	**a**	
		$\mathbf{a}_2\times$G1466	α	—	**a***	*rme* **a**/*rme* **a***
		$\mathbf{a}_3\times$G1466	α	—	**a***	
		$\mathbf{a}_4\times$G1466	*nm*	+	**a**	
V	**a**	$\mathbf{a}_1\times$G1466	α	—	**a***	
		$\mathbf{a}_2\times$G1466	α	—	**a***	*rme* **a**/*rme* **a***
		$\mathbf{a}_3\times$G1466	*nm*	+	**a**	
		$\mathbf{a}_4\times$G1466	*nm*	+	**a**	
	α	$\alpha_1\times$320	*nm*	+	*rme* α	
		$\alpha_1\times$17–15	α	+		*rme* α/*rme* α
		$\alpha_2\times$320	*nm*	+	*rme* α	
		$\alpha_2\times$17–15	α	+		
		$\alpha_3\times$320	*nm*	+	*rme* α	
		$\alpha_3\times$17–15	α	+		
		$\alpha_4\times$320	*nm*	+	*rme* α	
		$\alpha_4\times$17–15	α	+		

The genotypes of the different strains are: G1466 = *RME* α, 320 = *rme* **a**, 17–15 = *rme* **a***.

analysis for the **a**-mater sporulating F_1 progeny are given in Table A1. Both **a**-mater F_1 progeny gave only **a**-mater F_2 spores. Testing whether their genotypes were **a** or **a*** by mating gave a segregation of 2**a**:2**a*** in both F_1 diploids, which means that the F_1 diploid was of *rme* **a**/*rme* **a*** genotype (see Table A2).

The type V tetrad of strain **a*a***aα segregated 1**a** *spo*$^-$: 1**a** *spo*+ : 1α *spo*+ : 1*nm spo*$^-$, and this segregation by itself could not be explained directly by our model. Tetrad analysis for the sporulating F_1 progeny is given in Table A1. It shows that the **a**-mater F_1 segregant produced only **a**-mater spores and the α-mater F_1 segregant gave rise to 4 α spores. Mating and sporulation of diploids that were obtained by the F_2 segregants are given in Table A2. It shows that the

TABLE A3

Tetrad analysis of F_1 segregants from tetrads of strain **a*aa**α

F_1 tetrad type	Phenotype of F_1 segregants	No. of 4-spore tetrads examined	Segregation pattern	No. of 3-spore tetrads examined	Segregation pattern
II	*nm*	3	2**a**:2α	1	2**a**:1α
III	*nm*	8	2**a**:2α	1	1**a**:2α
	α	4	2**a**:2α	2	1**a**:2α

Note: Segregation patterns relate to mating phenotypes.

a-mater F_1 diploid segregated 2**a**:2**a***. The α-mater F_1 progeny segregated *rme* α spores. Thus the original tetrad (type V) contained an $\alpha\alpha$ segregant, meaning that it could not have arisen from bivalent pairing, but must have resulted from quadrivalent pairing. (Quadrivalent pairing is expected to occur occasionally in tetraploid strains; ROMAN, PHILLIPS and SANDS 1955). The other two spores could be *RME* **a**/*RME* **a*** and *rme* **a***/*rme* **a***. These genotypes have not been isolated before and therefore their assignment is only tentative. We know that **a*** haploids mate, though less efficiently than **a**; it is possible that an *rme* **a***/*rme* **a*** diploid cannot mate, and sporulation in **a***/**a*** may not be restored by *rme*.

TABLE A4

Mating-type and sporulation ability of different zygotes from F_2 segregants of tetrads of strain **a*aa**α

F_1 tetrad type	Phenotype of F_1 segregant	Mating of F_2 spore	Mating type of resulting zygote	Sporulation of zygote	Genotype of F_2 segregant	Genotype of F_1 segregant
II	*nm*	$\mathbf{a}_1\times$G1466	*nm*	+	**a**	
		$\mathbf{a}_2\times$G1466	*nm*	+	**a**	*rme* **a**/*RME* α
		$\alpha_3\times$320	*nm*	+	α	
		$\alpha_3\times$17–15	α	—	*RME* α	
		$\alpha_4\times$320	*nm*	+	α	
		$\alpha_4\times$17–15	α	+	*rme* α	
III	*nm*	$\mathbf{a}_1\times$G1466	*nm*	+	**a**	
		$\mathbf{a}_2\times$G1466	*nm*	+	**a**	*RME* α/*RME* **a**
		$\alpha_3\times$320	*nm*	+	α	
		$\alpha_3\times$17–15	α	—	*RME* α	
		$\alpha_4\times$320	*nm*	+	α	
		$\alpha_4\times$17–15	α	—	*RME* α	
	α	$\mathbf{a}_1\times$G1466	α	—	**a***	
		$\mathbf{a}_2\times$G1466	α	—	**a***	*rme* **a***/*rme* α
		$\alpha_3\times$320	*nm*	+	α	
		$\alpha_3\times$17–15	α	+	*rme* α	
		$\alpha_4\times$320	*nm*	+	α	
		$\alpha_4\times$17–15	α	+	*rme* α	

The genotypes of the different strains are: G1466 = *RME* α, 320 = *rme* **a**, 17–15 = *rme* **a***.

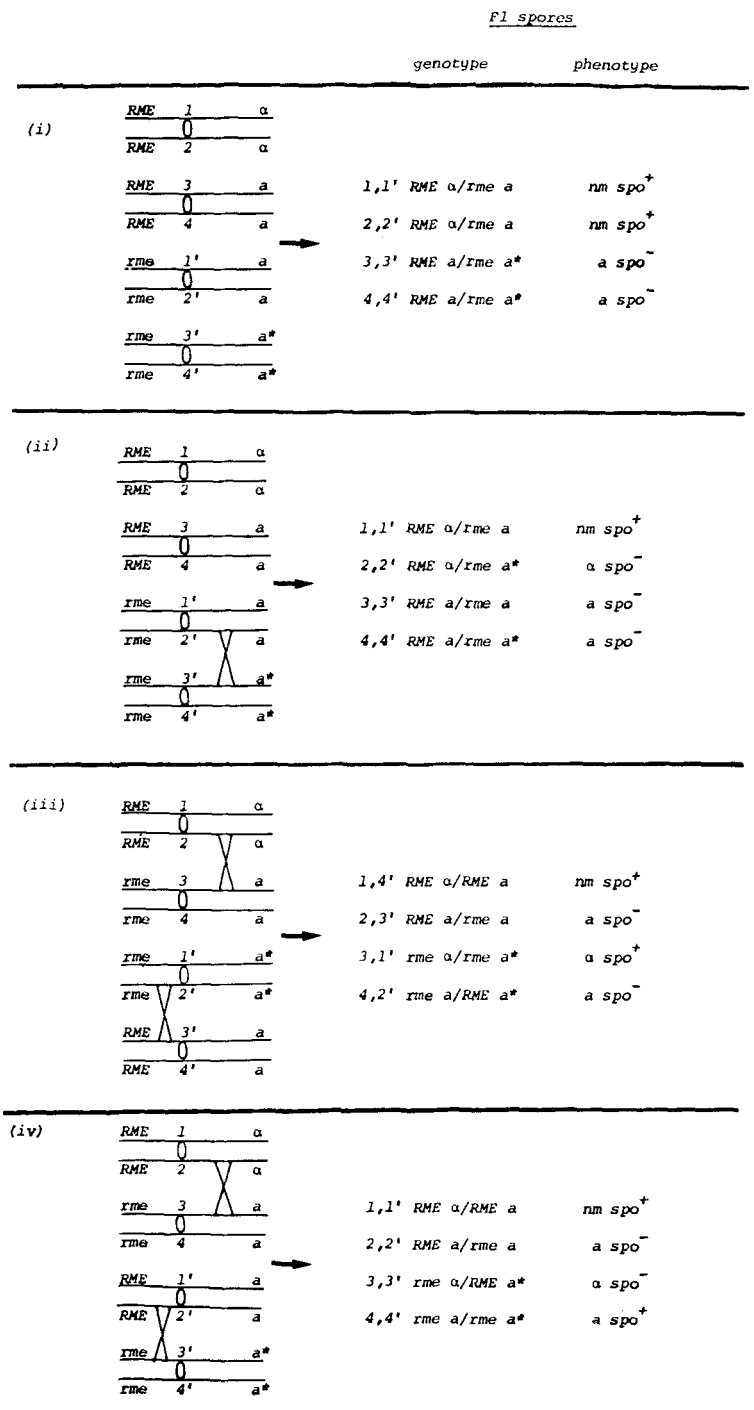

FIGURE A2.—Illustration of pairing and crossing over in the teraploid strain **a*aa**α. (i) Type I tetrad. (ii) Type II tetrad. (iii) Type III tetrad. (iv) Type IV tetrad. The numbers indicate centromeres.

The single type II tetrad of strain **a*a*a**α, which segregates 2**a** *spo*$^-$: 1α *spo*$^-$: 1α *spo*+, cannot be explained by our model, because we expect only two *spo*$^-$ segregants. The only way to explain it is by assuming that two asci of two spores each were isolated as one tetrad.

Type III tetrad of strain **a*aa**α gave segregation patterns of 2**a** *spo*$^-$: 1*nm spo*+ : 1α *spo*+. Results of tetrad analysis for the F_1 sporulating progeny are given in Table A3. Both the *nm* and α-mater F_1 diploid progeny segregated 2**a**:2α. Mating of F_2 segregants, as shown in Table A4, suggests that the *nm* spore is of *RME* α/*RME* **a** genotype and the α-mater F_1 segregant is *rme* **a***/*rme* α. This type of segregation is expected from bivalent pairing and crossing over as described in Figure A2 (iii).

Type II tetrad of strain **a*aa**α gave segregation pattern of 2**a** *spo*$^-$: 1α *spo*$^-$: 1*nm spo*+. Tetrad analysis of the *nm* segregant is given in Table A3, showing that it segregated 2**a**:2α. Analysis of diploids resulting from mating the F_2 segregants to strains G1466, 320 and 17–15 are given in Table A4. The results indicate that the F_1 diploid *RME* α/*rme* **a** gave rise to four types of spores: *RME* α, *rme* α, *RME* **a**, *rme* **a**. The pairing expected to give type II tetrad is illustrated in Figure A2 (ii).

Type I tetrad of **a*aa**α segregated as illustrated in Figure A2(i) to give rise to 2*nm spo*+ : 2**a** *spo*$^-$ spores.

Type IV tetrad of **a*aa**α segregated 1**a** *spo*$^-$: 1**a** *spo*+ : 1*nm spo*+ : 1α *spo*$^-$ by the pairing and crossovers as described in Figure A2 (iv).

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Corresponding editor: R. E. ESPOSITO